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AGRONOMIC PERFORMANCE OF SUGARCANE INOCULATED WITH *Nitrospirillum amazonense* (BR11145)[1]

VERÔNICA MASSENA REIS[2], FABIANO APARECIDO RIOS[3]*, GUILHERME BRAGA PEREIRA BRAZ[4], JAMIL CONSTANTIN[3], ERIC SEIJI HIRATA[5], DENIS FERNANDO BIFFE[3]

ABSTRACT - Sugarcane cultivation areas are usually characterized by low soil fertility and high nutrient demand, especially for nitrogen. Technologies that can raise crop yield and lower the use of pesticides and fertilizers are among the main needs of this crop. The objective of this study was to evaluate the agronomic performance of sugarcane after in-furrow application of the diazotrophic bacterium *Nitrospirillum amazonense* (BR11145). The treatments consisted of applying five doses of *N. amazonense*-based product to the planting furrow, 0.25, 0.5, 1.0, 1.5, and 2.0 L commercial product (c.p.) ha^{-1}; a treatment consisting of the combination of *Bacillus subtilis* and *Bacillus licheniformis*; one control without fertilization; and one control with fertilization. Based on the results obtained from the pooled analysis of two field experiments, the *N. amazonense*-based product improved the growth parameters of sugarcane plants, and at doses of 1.0 and 1.5 L c.p. ha^{-1}, these benefits resulted in a significant increase in yield and in the amount of sugar per hectare. Inoculation with diazotrophic bacteria can benefit sugarcane, especially because it promotes the growth and yield of the crop similarly to fertilizers.

Keywords: Diazotrophic bacteria. Associative nitrogen fixation. Growth promotion. *Saccharum officinarum*.

PERFORMANCE AGRONÔMICA DA CANA-DE-AÇÚCAR SUBMETIDA À INOCULAÇÃO DE *Nitrospirillum amazonense* (BR11145)

RESUMO - As áreas de cultivo de cana-de-açúcar apresentam em sua maioria, baixa fertilidade do solo e, elevada demanda por nutrientes, especialmente o nitrogênio. Tecnologias que possam contribuir com o aumento de produtividade, aliado ao menor uso de defensivos e fertilizantes estão entre as principais necessidades da cultura. Objetivou-se avaliar o desempenho agronômico da cana-de-açúcar após a aplicação em sulco de plantio da bactéria diazotrófica *Nitrospirillum amazonense* (BR11145). Os tratamentos foram compostos pela aplicação no sulco de plantio de cinco doses de produto à base de *N. amazonense*: 0,25, 0,5, 1,0, 1,5 e 2,0 L p.c. ha^{-1}, um tratamento composto pela associação entre as bactérias *Bacillus subtilis* e *Bacillus licheniformis*, além uma testemunha sem adubação e outra com adubação. Com base nos resultados obtidos da análise conjunta de dois experimentos, pode-se afirmar que: o produto à base de *N. amazonense* proporciona melhorias nos parâmetros de crescimento das plantas de cana-de-açúcar, sendo que, nas doses de 1,0 e 1,5 L p.c. ha^{-1}, estes benefícios resultaram em aumento significativo de produtividade e na quantidade de açúcar por hectare. A inoculação de bactérias diazotróficas representa um benefício para a cana-de-açúcar, especialmente por favorecer o crescimento e a produtividade da cultura similarmente à utilização de fertilizantes.

Palavras-chave: Bactérias diazotróficas. Fixação associativa de nitrogênio. Promoção de crescimento. *Saccharum officinarum*.

*Corresponding author
[1]Received for publication in 02/28/2020; accepted in 07/09/2020.
[2]National Center of Agrobiology Research, Embrapa Agrobiologia, Seropédica, RJ, Brazil; veronica.massena@embrapa.br – ORCID: 0000-0003-2149-7725.
[3]Agricultural Science Department, Universidade Estadual de Maringá, Maringá, PR, Brazil; fabianoap.rios@gmail.com – ORCID: 0000-0003-1431-074X, jamil.constantin@gmail.com – ORCID: 0000-0001-6610-699X, denisbiffe@gmail.com – ORCID: 0000-0002-2036-3429.
[4]Agricultural Science Department. Universidade de Rio Verde, Rio Verde, GO, Brazil; guilhermebrag@gmail.com – ORCID: 0000-0002-0396-7140.
[5]Basf S/A, Santo Antônio de Posse, SP, Brazil; eric.hirata@basf.com - ORCID: 0000-0002-4550-833X.

INTRODUCTION

In Brazil, sugarcane has high socioeconomic importance, especially due to its ability to be cultivated in all regions of the country. For the 2019/2020 harvest, the expected average yield of Brazilian sugarcane fields is 74.21 t ha^{-1} (CONAB, 2019). Regarding the productive potential of this crop, the national average is below what can be achieved based on the genetics of the varieties present in the market. The factors that influence these yield variations include soil and climatic conditions of its cultivation and management practices.

When correlating the soil factor, especially the soil fertility level, with management practices, the need to improve the nutrient supply to sugarcane to obtain higher yields has been shown. In this sense, macronutrients are the elements required in the greatest amounts by plants, including nitrogen, which is essential for plant metabolism because it directly participates in the synthesis of proteins and chlorophylls (ANDRADE et al., 2003). In sugarcane, the nutritional requirement for nitrogen is high, and under deficient conditions, plants may show reductions in yield and longevity, leading to a decrease in the number of sugarcane cuts (VITTI et al., 2007).

To avoid losses in sugarcane yield due to nitrogen deficiency, the most commonly used management practice is to apply chemical fertilizers. On average, the doses of nitrogen fertilizers used in Brazilian sugarcane plantations are 40 and 80 kg of nitrogen ha^{-1} for plant cane and ratoon cane, respectively (NUNES JÚNIOR et al., 2005). However, chemical fertilization in sugarcane plantations sometimes has low agronomic efficiency (FRANCO et al., 2008).

Another tool that has been widely studied in recent years is inoculation with diazotrophic bacteria, which increase the supply of nitrogen to the this crop and promote its growth (URQUIAGA; CRUZ; BODDEY, 1992; SCHULTZ et al., 2014, 2016). Diazotrophic bacteria supply this nutrient through biological nitrogen fixation, in which microorganisms reduce atmospheric nitrogen to ammonia. Diazotrophic bacteria can also solubilize phosphates and zinc and produce phytohormones (SANTI; BOGUSZ; FRANCHE, 2013).

Despite the good results obtained from inoculation with diazotrophic bacteria in sugarcane (BODDEY et al., 2003; BENEDUZI et al., 2013), there are still questions about how to increase the agronomic efficiency of this process and maximize the positive interaction between the microorganism and the crop. Schultz et al. (2012), in an experiment evaluating the efficiency of inoculation with diazotrophic bacteria in sugarcane, found that the response of the crop differed by variety. In a study conducted by Oliveira et al. (2006), it was found that the sugarcane soil environment also influenced the efficiency of inoculation with diazotrophic bacteria, especially in low-fertility soils.

Among the diazotrophic and growth-promoting bacteria, *Azospirillum* species have been the most studied, especially the species *A. brasilense* and *A. lipoferum* (CASSÁN; VANDERLEYDEN; SPAEPEN, 2014). The species *Nitrospirillum amazonense*, previously classified as *Azospirillum amazonense* (LIN et al., 2014), is a bacterium that can fix nitrogen and in many other ways promote the growth of the plants it colonizes. In other studies, growth promotion, regardless of the contribution to nitrogen supply, was responsible for the increased mass and sugar content of sugarcane varieties inoculated with a mix of five bacterial strains, including *N. amazonense* (SCHULTZ et al., 2014, 2016).

In this context, the present study aimed to evaluate the growth, development, and yield of sugarcane after in-furrow application of the diazotrophic bacterium *N. amazonense* (BR11145).

MATERIALS AND METHODS

Two experiments were conducted in the municipalities of Tapejara, state of Paraná (23°49′ 47.74″S and 52°53′22.94″W) and Quatá, state of São Paulo (22°13′03.20″S and 50°34′42.22″W), respectively. Both experiments were conducted in a commercial sugarcane plantation in June 2016 and July 2017.

The soil samples used for physicochemical characterization were collected at a depth of 0 to 0.2 m, and the results are presented below. In the Tapejara experiment, the values were pH (water) = 6.3, P = 59 mg dm^{-3}, H + Al = 1.5 $cmol_c$ dm^{-3}, Ca = 1.8 $cmol_c$ dm^{-3}, Mg = 0.7 $cmol_c$ dm^{-3}, K = 0.1 $cmol_c$ dm^{-3}, OM = 12 g kg^{-1}, clay = 198 g kg^{-1}, silt = 35 g kg^{-1}, and sand = 767 g kg^{-1} (sandy loam texture). In the experiment conducted in Quatá, we found pH (water) = 6.8, P = 12 mg dm^{-3}, H + Al = 1.2 $cmol_c$ dm^{-3}, Ca = 1.5 $cmol_c$ dm^{-3}, Mg = 0.8 $cmol_c$ dm^{-3}, K = 0.16 $cmol_c$ dm^{-3}, OM = 12 g kg^{-1}, clay = 191 g kg^{-1}, silt = 40 g kg^{-1}, and sand = 769 g kg^{-1} (sandy loam texture).

According to the Köppen classification, the predominant climate in the municipality of Tapejara is subtropical, with hot summers and rare frosts, rainfall usually concentrated in the summer months, and no well-defined dry season (Cfa). The predominant climate of Quatá is subtropical, with dry winters and hot summers (Cwa) (CLIMATE-DATA, 2019). The mean temperatures and precipitation recorded during the experiments are shown in Figure 1.

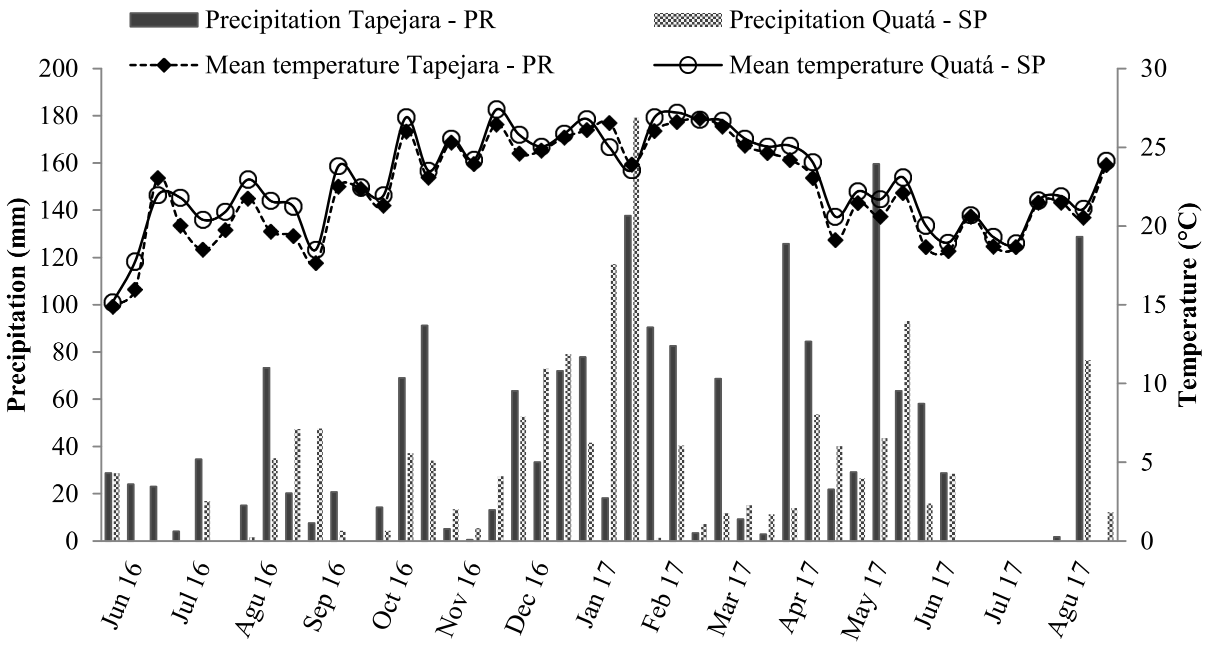

Figure 1. Mean temperature and cumulative precipitation during the experiments performed with sugarcane inoculated with diazotrophic bacteria.

In both experiments, a randomized block design was used, with eight treatments and five replicates. The treatments were applied in the furrow and on sugarcane cuttings and consisted of five doses of a commercial *N. amazonense*-based product (0.25, 0.5, 1.0, 1.5, and 2.0 L commercial product (c.p.) ha^{-1}), a fertilized control, an absolute control (without fertilization and without inoculation), and a treatment consisting of the combination of *Bacillus subtilis* and *Bacillus licheniformis* at a dose of 1.0 kg c.p. ha^{-1}. The *N. amazonense*-based product used in the present study had a concentration of 1.0 × 10^{8} CFU mL^{-1}, with the brand name Aprinza® (EMBRAPA, 2018). The product with *B. subtilis* and *B. licheniformis* (Nemix C® (FMC, 2019)) had a concentration of 1.6 × 10^{10} CFU g^{-1} of each species, and the commercial formulation. In both controls without inoculation, the plants were sprayed only with water to provide similar conditions to the other treatments in which the bacteria were inoculated. The experimental units were composed of six 10-m-long sugarcane rows, totaling an area of 72 m^2. In the evaluations and at harvest, 1.0 m from each end of the experimental units and a border line were disregarded, totaling a used area of 38.4 m^2.

The areas were prepared by performing heavy harrowing, followed by intermediate and light harrowing, opening the furrows at a depth of approximately 0.5 m. Sugarcane was planted manually in a conventional system. In both sites, the planting was performed in a double-row system. The spacing was 0.9 m within each double row and 1.5 m between the double interrows. Except for the experimental units from the control without fertilization, in all other treatments, doses of 25 kg ha^{-1} of N, 60 kg ha^{-1} of P_2O_5, and 60 kg ha^{-1} of K_2O were applied to the planting furrow in Tapejara. The numbers were and 40 kg ha^{-1} of N, 40 kg ha^{-1} of P_2O_5, and 60 kg ha^{-1} of K_2O in Quatá. At planting, stem cuttings were used that contained approximately three buds of the variety RB 96-6928, which were placed in the furrow, totaling approximately 21 buds per linear meter.

For applying the treatments, a CO_2 constant-pressure backpack sprayer was used, which was equipped with a TP-110.03 fan nozzle under a pressure of 196,133 Pa. These application conditions provided the equivalent of 150 L ha^{-1} of spray solution. The treatment solutions were made (with chlorine-free water) minutes before they were applied. At the end of the application of each treatment, the sprayer set was washed with water. In both experiments, the treatments were applied in the planting furrow, and spraying was performed on the furrow and the sugarcane cuttings. Then they were covered with soil. During the application, the soil was moist, and the sky had few clouds. The relative humidity was 72% and 69%, the temperature was 24 °C and 20 °C, and the wind speed was 4.32 ms^{-1} and 4.32 ms^{-1} for the experiments conducted in Tapejara and Quatá, respectively.

To measure the effects of the treatments, both experiments evaluated the following response variables at 60, 90, and 180 days after planting (DAP) and at harvest: insertion height of the last expanded leaf (mean of 15 previously identified plants per experimental unit), number of tillers in 5 m of the two central rows of plants of the experimental unit (presented as the mean for 1 linear meter). In addition, at 90 DAP, leaf area and shoot dry mass were evaluated by collecting data from 15 plants per experimental unit. The leaf area (cm^2) was evaluated using an LI 3100 leaf area meter. At 180 DAP and at harvest, the number of internodes per plant was counted, and the lengths of three internodes located in the middle third of each plant were measured. Additionally, on those same dates, stem diameter was measured on an internode located in the middle third. All of these evaluations were performed on the same 15 plants per experimental unit.

The macronutrient contents (nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur) in the leaf blade (without vein) of the +1 leaf were evaluated at 60, 90, and 180 DAP. In addition, at 90 DAP, the macronutrient levels in the sugarcane shoot (leaf + stem) were evaluated. Nitrogen was evaluated with the semimicro-Kjeldahl apparatus, phosphorus by metavanadate colorimetry, potassium by emission flame photometry, sulfur by barium sulfate turbidimetry, and calcium and magnesium by atomic-absorption spectrophotometry. After collecting the material, the leaf and shoot samples of the plants were sent to a laboratory for conducting all the analytical processes relevant to this study. The methods for nutritional analysis and the sampling procedures were the same as those proposed by CONSECANA (2006). For the nutritional evaluations, 20 and 15 plants were sampled per experimental unit to determine their leaf and shoot contents, respectively.

In both experiments, the harvest was performed manually at the beginning of July 2017, by cutting the stalks present in the useful area of the experimental unit, and the total weight was later converted to yield per hectare (t ha^{-1}). The amount of sugar per ton of sugarcane was quantified (CONSECANA, 2006) and extrapolated to kilograms of sugar per hectare based on yield.

The data were analyzed with R software. The variance of each response variable was subjected to homogeneity analysis by applying the Bartley test to each variable in each experiment separately (PIMENTEL-GOMES, 2000). The principle of homogeneity was met, so the pooled analysis of the experiments was carried out. Tukey's test (threshold $p \leq 0.10$) was used to compare the treatment means.

RESULTS AND DISCUSSION

Of all the response variables analyzed, the yield and the amount of sugar per hectare showed an interaction between treatment and site in the pooled analysis. The means of these variables were analyzed separately by treatment within each site. For the other variables, the data were analyzed considering only the main effect of the treatment (mean of the two sites). The results presented refer to the comparisons of means that were significantly different on analysis of variance.

The height of the sugarcane tillers was influenced only by the effect of the treatment in the evaluation performed at the time of harvest, at which time the *N. amazonense* groups at doses of 0.5 L c.p. ha^{-1} and above and the *B. subtilis* + *B. licheniformis* group had grown higher than the unfertilized, uninoculated control (Table 1). Increases in the height of the sugarcane tillers are important from a productive standpoint, since taller plants tend to have larger reserves and therefore greater yield. Despite this, the increase in height, with maintenance or reduction of stem diameter, may provide greater susceptibility to lodging of sugarcane plants (OLIVEIRA; BRAGA, 2011).

Regarding sugarcane tillering, although differences were observed in the first evaluation (60 DAP), due to the ambiguity of the test comparing means, it was only possible to say that *N. amazonense* at the dose of 0.25 L ha^{-1} resulted in higher tillering than *B. subtilis* + *B. licheniformis* (1.0 L ha^{-1}). On the other hand, in the evaluation at harvest, sugarcane plants treated with *N. amazonense* at doses equal to or greater than 1.0 L c.p. ha^{-1} showed more tillers than the treatment without fertilization or inoculation. To get a sense of the magnitude of the benefit generated by the use of *N. amazonense* at the aforementioned doses on the tillering of the crop, plants that received these treatments presented, on average, 13% more tillers than those grown in the control experimental units without fertilization or inoculation. Among the components of the sugarcane production potential, in addition to plant height and stem diameter, tillering (SILVA; CATO; COSTA, 2010) stands out, as treatments that increase the expression of this trait tend to exhibit higher yields.

Table 1. Biometric and agronomic variables of sugarcane subjected to inoculation with diazotrophic bacteria.

Treatment (L or kg ha^{-1})	Height of tillers (cm)	Number of tillers		LA (cm^2)	NI	LI (mm)	SD (mm)	
	Harvest	60[1/]	Harvest	90[1/]	Harvest	Harvest	180[1/]	Harvest
Control	241.1 c	3 ab	11.1 b	203.7 b	21.1 b	36.3 b	18 b	24.3 b
Fertilizer control	255.2 bc	3 ab	12 ab	233.5 ab	21.8 ab	38 ab	19.9 a	25.5 ab
N. amazonense (0.25)	255.1 bc	3.9 a	12.2 ab	236.4 ab	22.4 ab	38 ab	20.5 a	25.4 ab
N. amazonense (0.5)	262.3 ab	2.9 ab	12.1 ab	229.4 ab	22.9 a	39.1 ab	20.5 a	26.4 a
N. amazonense (1.0)	273.5 a	2.9 ab	12.7 a	254.8 ab	23.5 a	39.7 a	20.7 a	26.7 a
N. amazonense (1.5)	266.2 ab	2.6 b	12.6 a	277.1 a	23.6 a	39.3 a	21 a	26.8 a
N. amazonense (2.0)	269.7 ab	2.9 ab	12.5 a	269.2 ab	23.2 a	39.5 a	20.6 a	26.2 a
B. subtilis + *B. licheniformis* (1.0)	259.1 ab	2.6 b	11.9 ab	270.6 a	22.3 ab	38.9 ab	20.3 a	25.5 ab
CV (%)	5	30.6	7.6	21.1	6.2	5.9	5.7	4.2

[1/]DAP: days after planting. LA: leaf area; NI: number of internodes; LI: length of internodes; SD: stem diameter. Means followed by the same letter in a column do not differ by Tukey's test ($p \leq 0.10$).

Still regarding the growth parameters of the sugarcane, at 90 DAP, the leaf area was greater in the plants subjected to the in-furrow application of the *N. amazonense*-based product at a dose of 1.5 L c.p. ha^{-1} or *B. subtilis* + *B. licheniformis* than in those that did not receive fertilization or inoculation (Table 1). On the same date, no differences were observed in the shoot dry mass accumulation of sugarcane plants inoculated with diazotrophic bacteria (data not shown).

At 180 DAP, no difference was observed in the number of internodes of the sugarcane plants as a function of treatment (Table 1). On the other hand, at the time of the crop harvest, the sugarcane plants that developed in soil with application of the *N. amazonense*-based product at doses starting at 0.5 L c.p. ha^{-1} on the day of planting showed more internodes than the control without fertilization or inoculation (Table 1). Similar behavior was observed for the length of internodes (LI), where doses of 1.0 or higher resulted in higher values of this variable than the absolute control did.

Stem diameter at 180 DAP was increased by all treatments relative to the control without fertilization or inoculation, regardless of the dose (Table 2). A fact that deserves to be highlighted in this evaluation is that the performance of the treatments with bacterial inoculation was similar to the performance in the chemical fertilization control, demonstrating that in certain situations, it is possible to opt for this type of management without negatively affecting the crop. At harvest time, differences were observed in the stem diameter of sugarcane plants, and those that developed in soil with application of *N. amazonense*-based product in doses starting at 0.5 L c.p. ha^{-1} showed higher values than those that were not fertilized or inoculated at the time of planting.

As for the leaf macronutrient contents, no differences were observed in the accumulation of magnesium or sulfur in the tissue of sugarcane plants in any of the evaluations performed (data not shown). On the other hand, nitrogen in leaves at 90 and 180 DAP in the *N. amazonense* groups was higher than in the control group without fertilization, especially when the *N. amazonense* dose was 1.5 or 2.0 L c.p. ha^{-1} (Table 2).

Regarding nitrogen specifically, a fact that deserves attention is that at 90 DAP, the plants treated with the two highest doses of *N. amazonense* had a higher concentration of this macronutrient than the plants treated with chemical fertilizer. This superiority might have been related to the fact that diazotrophic bacteria can supply nitrogen in an associative manner to certain plant species, compensating for possible losses related to the inefficiency of the application of chemical fertilizer, such as due to leaching of the element (FRANCO et al., 2008).

As for phosphorus, at 90 DAP, only the dose of 1.5 L c.p. ha^{-1} of the *N. amazonense*-based product provided a greater leaf concentration than no fertilization or inoculation (Table 2). In the last evaluation of the leaf concentration of this macronutrient, in addition to the 1.5 L c.p. ha^{-1} treatment, the *N. amazonense*-based product at a dose of 0.5 L c.p. ha^{-1} also promoted an increase relative to the control without fertilization. The action of *N. amazonense* in increasing leaf phosphorus levels may be related to the ability of some diazotrophic bacteria to help solubilize phosphates in the soil (SHUKLA et al., 2008), increasing the availability of this macronutrient to the plants.

Table 2. Leaf nitrogen, phosphorus, potassium, and calcium concentrations in sugarcane inoculated with diazotrophic bacteria.

Treatment (L or kg ha^{-1})	N (%)			P (%)			K (%)	Ca (%)
	60[1/]	90	180	60	90	180	180	90
Control	2.3 ab	2.1 b	1.2 b	0.4 ab	0.30 b	0.27 c	1.7 b	0.6 b
Fertilizer control	2.2 b	2 b	1.3 ab	0.4 ab	0.34 ab	0.28 bc	1.8 ab	0.6 b
N. amazonense (0.25)	2.5 a	2.1 ab	1.3 ab	0.4 ab	0.33 ab	0.3 abc	1.8 ab	0.6 b
N. amazonense (0.5)	2.5 a	2.1 ab	1.3 ab	0.4 ab	0.32 ab	0.31 ab	2 a	0.6 b
N. amazonense (1.0)	2.4 ab	2.2 ab	1.3 a	0.42 ab	0.34 ab	0.3 abc	2 a	0.7 ab
N. amazonense (1.5)	2.5 a	2.3 a	1.3 a	0.43 a	0.35 a	0.32 a	1.9 a	0.7 ab
N. amazonense (2.0)	2.3 ab	2.3 a	1.3 a	0.4 ab	0.35 ab	0.3 abc	1.8 ab	0.7 a
B. subtilis + *B. Licheniformis* (1.0)	2.3 ab	2.1 ab	1.3 ab	0.3 b	0.33 ab	0.27 c	1.8 ab	0.6 ab
CV (%)	8.5	7.7	4.9	8.1	10.8	9.7	9.97	14.1

[1/]DAP: days after planting. Means followed by the same letter in the same column do not differ by Tukey's test ($p \leq 0.10$).

For potassium and calcium, the effect of the treatments on the increase in their leaf concentrations over the effect of the control without fertilization was less pronounced, with differences being observed only at 180 DAP under doses of *N. amazonense* starting at 1.0 L c.p. ha^{-1} and at 90 DAP only at the highest dose of the product for each macronutrient. In addition to the leaf levels, at 90 DAP the nutritional composition of the entire shoot of the sugarcane plants was evaluated, and a clear effect of treatment was only observed for phosphorus (Table 3). Thus, the use of an *N. amazonense*-based product at a dose of 1.5 L c.p. ha^{-1} could provide more phosphorus to plant tissue than no fertilization or inoculation.

Table 3. Nutrient content in sugarcane plants inoculated with diazotrophic bacteria.

Treatment (L or kg ha^{-1})	Nutritional contents (%)					
	N	P	K	Ca	Mg	S
Control	2.14 ab	0.30 b	2.45	0.63	0.21	0.20 ab
Fertilizer control	2.07 b	0.32 ab	2.38	0.67	0.20	0.19 b
N. amazonense (0.25)	2.24 ab	0.32 ab	2.64	0.59	0.21	0.20 ab
N. amazonense (0.5)	2.26 ab	0.30 b	2.66	0.60	0.20	0.20 ab
N. amazonense (1.0)	2.25 ab	0.33 ab	2.52	0.63	0.20	0.22 a
N. amazonense (1.5)	2.34 a	0.34 a	2.42	0.68	0.20	0.20 ab
N. amazonense (2.0)	2.20 ab	0.32 ab	2.38	0.71	0.21	0.21 ab
B. subtilis + *B. Licheniformis* (1.0)	2.16 ab	0.33 ab	2.54	0.67	0.22	0.22 a
CV (%)	7.58	9.24	19.52	15.40	9.83	9.55

Means followed by the same letter in the same column do not differ by Tukey's test ($p \leq 0.10$). Ns: not significant by the F test.

All treatments evaluated provided increased sugarcane stem yield than the control in which fertilization and inoculation were not performed at the time of planting (Table 4). The use of the *N. amazonense*-based product at doses of 0.25, 0.5, and 2.0 L c.p. ha^{-1}, as well as the combination of *B. subtilis* and *B. licheniformis*, showed yield values similar to those recorded in the treatment with chemical fertilization without bacterial inoculation. On the other hand, the dose of 1.5 L c.p. ha^{-1} in the

Tapejara experiment provided a yield increase of 18.1 t ha^{-1}, while in the Quatá experiment, the dose of 1.0 L ha^{-1} resulted in an increase of 14.7 t ha^{-1} over the fertilized control, both significant. Regarding the amount of sugar per hectare, doses of 1.0 and 1.5 L c.p. ha^{-1} provided significant gains in both sites when compared to the respective fertilizer controls. In contrast, in another study, there was no increase in sugarcane yield when the stems were treated by immersion in a solution containing a mix of diazotrophic bacteria before planting (SIMÕES et al., 2019). The divergent results between studies may be due to differences in inoculation methods, varieties, soil type, and climate.

As mentioned above, research studies have shown a greater positive effect of inoculation with diazotrophic bacteria on sugarcane under conditions of low soil fertility (OLIVEIRA et al., 2006). In the present study, the soil conditions in which the experiments were conducted were adequate for the good development of the crop, and even in this scenario, sugarcane stem yield increased when the plants were inoculated with the diazotrophic bacteria. This suggests a very interesting potential for the use of this species in the sugarcane crop.

Table 4. Stem yield and sugar content after application of treatments containing diazotrophic bacteria in sugarcane crops.

Treatment	Yield (t ha $^{-1}$)		Sugar (kg ha^{-1})	
(L or kg ha^{-1})	Tapejara	Quatá	Tapejara	Quatá
Control	78.9 d	119.4 c	10800,8 c	16933,9 c
Fertilizer control	97.8 b	125.0 b	13687,7 b	17066,7 bc
N. amazonense (0.25)	103.1 bc	130 abc	14928,7 ab	18822,8 abc
N. amazonense (0.5)	102.9 bc	133.1 ab	14683,9 ab	19043,2 ab
N. amazonense (1.0)	112.1 ab	139.7 a	15811,9 a	20408,3 a
N. amazonense (1.5)	115.9 a	132 ab	16158,5 a	19223,5 a
N. amazonense (2.0)	106.2 abc	133.1 ab	14986,9 ab	19653,4 a
B. subtilis + *B. licheniformis* (1.0)	102.6 bc	129.4 abc	14282,8 ab	19043,4 ab
CV (%)	5.42		6.65	

Means followed by the same letter in the same column do not differ by Tukey's test ($p \leq 0.10$).

The amount of sugar produced per hectare was increased by all treatments compared to the control without fertilization, except for the lowest dose of *N. amazonense* in the Quatá experiment (Table 4). Moreover, doses of 1.0 and 1.5 L c.p. ha^{-1} in Tapejara and of 1.0, 1.5, and 2.0 L c.p. ha^{-1} in Quatá provided higher sugar levels than the fertilizer control. In the treatment consisting of the combination of species of the genus *Bacillus* sp., the results show that there were only increases in the sugar produced relative to that of the control without fertilization.

Regarding the technological analyses of the respective experiment, there was no effect of the treatments on its results, demonstrating that the use of *N. amazonense* did not provide significant changes in the agronomic performance of sugarcane (data not shown).

When considering the set of response variables at the time of harvest, there was a positive response to the use of *N. amazonense* via in-furrow application at the time of planting. Analyzing the gain provided relative to the treatment without fertilization, we observed that the *N. amazonense*-based product, when applied at doses of 1.0 L c.p. ha^{-1} or higher, provided a mean increase of 12% in the leaf area, 13.5% in the number of tillers, 11% in the number of internodes, 8.8% in the internode length, and 9% in the stem diameter. As a consequence of the gains in crop growth, the mean yield of these treatments was 41% higher than the control in Tapejara and 13% higher in Quatá. The differences in yield between sites show the importance of the interaction of the diazotrophic bacterium with different environments, even if the results were positive for different regions. Although the benefits of inoculation with diazotrophic bacteria for sugarcane are widely demonstrated, generally these results are obtained with inoculations of different bacterial species in mixtures (SCHULTZ et al., 2017; SIMÕES et al., 2019; MATOSO et al., 2020). This emphasizes the efficiency of one *N. amazonense* strain used alone, and this was the first study showing the benefit of using this crop treatment in sugarcane in two different sites.

Although biological nitrogen fixation has been the main explanation for the benefits of inoculation, it is possible that bacteria can stimulate ion absorption and nutrient transport in plants; however, the physiological explanation of the bacterial mixture is still little understood (SCHWAB; TERRA; BALDANI, 2018; SANTOS et al., 2019). Reis Junior et al. (2008) found no increase in nitrogen accumulation by maize plants after inoculation with *Azospirillum amazonensis*. In two sugarcane varieties, inoculation with five bacterial species did not result in higher nitrogen accumulation in the shoots of the plants (SCHULTZ et al., 2017). As the increase in root apparatus and plant biomass has been the main consequence of inoculation, the increase in nutrient levels may be related to the efficiency of their use by plants, which strongly justifies the adoption of this crop management practice.

The potential of *N. amazonense* to be used in sugarcane management indicates that this diazotrophic bacterium can help address the problem noticed by the scientific community of the unsustainability of the use of nitrogen fertilizers in this crop. One of the benefits of the commercial exploitation of sugarcane is related to the production of ethanol, which pollutes less than fossil fuels (FINOTTI et al., 2009), and its increased use leads to lower use of fossil fuels. On the other hand, large quantities of fossil fuels are used to produce nitrogen fertilizers (LAGREID; BOCKMAN; KAARSTAD, 1999). In this sense, researchers have questioned the extent to which sugarcane production is sustainable when using high amounts of chemical nitrogen fertilizers. In this context, the inoculation of *N. amazonense* can help reduce the use of nitrogen fertilizers without affecting the productive potential of the crop.

CONCLUSIONS

The diazotrophic bacterium *N. amazonense* can improve the growth and development parameters of sugarcane plants, increasing the height and number of tillers, the length and number of internodes, stem diameter, and yield, without changing the technological parameters. The *N. amazonense*-based product, applied per planting furrow, promoted a significant increase in sugar yield at the doses of 1.0, 1.5, and 2.0 L c.p. ha^{-1}, whereas the sugarcane stem yield was significantly higher than in the fertilized control without inoculation when the doses of 1.0 and 1.5 L c.p. ha^{-1} were used, meaning it is more reliable in the field.

REFERENCES

ANDRADE, A. C. et al. Adubação nitrogenada e potássica em capim-elefante (*Pennisetum purpureum* schum. cv. napier). **Ciência e Agrotecnologia**, 27: 1643-1651, 2003.

BENEDUZI, A. et al. Diversity and plant growth promoting evaluation abilities of bacteria isolate from sugarcane cultivated in the South of Brazil. **Applied Soil Ecology**, 63: 94-104, 2013.

BODDEY, R. M. et al. Endophytic nitrogen fixation in sugarcane: present knowledge and future applications. **Plant and Soil**, 252: 139-149, 2003.

CASSÁN, F.; VANDERLEYDEN, J.; SPAEPEN, S. Physiological and agronomical aspects of phytohormone production by model plant-bacteria-promoting rhizobacteria (PGPR) belonging to the genus *Azospirillum*. **Journal Plant Growth Regulation**, 33: 440-459, 2014.

CLIMATE-DATA. **Clima Brasil, 2019**. Disponível em:. <https://pt.climate-data.org/america-do-sul/brasil-114/>. Acesso em: 16 dez. 2019.

COMPANHIA NACIONAL DE ABASTECIMENTO - CONAB. **Acompanhamento da safra brasileira:, Segundo levantamento da safra de cana-de-açúcar, 2019/2020**. 2019. Brasília. Disponível em:. <https://www.conab.gov.br/info-agro/safras/cana/boletim-da-safra-de-cana-de-acucar> Acesso em: 16 dez. 2019.

CONSELHO DE PRODUTORES DE CANA-DE-AÇÚCAR, AÇÚCAR E ÁLCOOL DO ESTADO DE SÃO PAULO - CONSECANA. **Manual de Instruções**. 5 ed. Piracicaba, SP: CONSECANA, 2006. 112 p.

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA - EMBRAPA. **Inoculante para fixação de nitrogênio para cana é lançado pela Basf e Embrapa**. 2018. Disponível em: <https://www.embrapa.br/en/busca-de-30 noticias/-/noticia/39688081/inoculante-para-fixacao-de-nitrogenio-para-cana-e-lancado-pela-basf-e-embrapa> Acesso em: 16 dez. 2019.

FINOTTI, A. R. et al. Avaliação da influência do etanol sobre o grau de volatilização BTEX em solos impactados por derrames de gasolina/etanol. **Engenharia Sanitaria e Ambiental**, 14: 443-448, 2009.

FOOD MACHINARY CORPORATION - FMC. **Biológicos - Nemix C**. Disponível em: <http://m.fmcagricola.com.br/produtos/detalhes/157> Acesso em: 18 dez. 2019.

FRANCO, H. C. J. et al. Aproveitamento pela cana-de-açúcar da adubação nitrogenada de plantio. **Revista Brasileira de Ciência do Solo**, 32: 2763-2770, 2008.

LAGREID, M.; BOCKMAN, O. C.; KAARSTAD,

O. **Agriculture, fertilizers and the environment**. Cambridge: CABI. 1999, 294 p.

LIN, S. Y. et al. Description of *Niveispirillum* fermenti gen. nov., sp. nov., isolated from a fermentor in Taiwan, transfer of *Azospirillum irakense* (1989) as *Niveispirillum irakense* comb. nov., and reclassification of *Azospirillum amazonense* (1983) as *Nitrospirillum amazonense* gen. nov. **Antonie Van Leeuwenhoek**, 105: 1149-1162, 2014.

MATOSO, E. S. et al. Influência do uso de um mix de bactérias diaztróficas na biometria e no conteúdo de clorofila de plantas de cana-de-açúcar. **Brazilian Journal of Development**, 6: 7261-7274, 2020.

NUNES JÚNIOR, D. et al. **Indicadores agrícolas do setor canavieiro: safra 2003/2004**. 57. ed. Ribeirão Preto, SP: IDEA, 2005. 111 p.

REIS JUNIOR, F. B. et al. Inoculação de *Azospirillum amazonense* em dois genótipos de milho sob diferentes regimes de nitrogênio. **Revista Brasileira de Ciência do Solo**, 32: 1139-1146, 2008.

OLIVEIRA, A. R.; BRAGA, M. B. **Florescimento e acamamento de cultivares de cana-de-açúcar submetidas a diferentes lâminas de irrigação**. Petrolina, PE: Embrapa Semiárido, 2011. 23 p. (Boletim de Pesquisa e Desenvolvimento, 87).

OLIVEIRA, A. L. M. et al. Yield of micropropagated sugarcane varieties in different soil types following inoculation with endophytic diazotrophic bacteria. **Plant and Soil**, 284: 23-32, 2006.

PIMENTEL-GOMES, F. P. **Curso de estatística experimental**. 11. ed. São Paulo, SP: Nobel, 2000. 466 p.

SANTOS, S. et al. Rooting and growth of pre-germinated sugarcane seedlings inoculated with diazotrophic bacteria. Applied Soil Ecology, 133: 12 -23, 2019.

SANTI, C.; BOGUSZ, D.; FRANCHE, C. Biological nitrogen fixation in non-legume plants. **Annals of Botany**, 111: 743-767, 2013.

SCHWAB, S.; TERRA, L. A.; BALDANI, J. I. Genomic characterization of *Nitrospirillum amazonense* strain CBAmC, a nitrogen-fixing bacterium isolated from surface-sterilized sugarcane stems. **Molecular Genetics and Genomics**, 293: 997-1016, 2018.

SCHULTZ, N. et al. Avaliação agronômica de duas variedades de cana-de-açúcar inoculadas com bactérias diazotróficas e adubadas com nitrogênio. **Pesquisa Agropecuária Brasileira**, 47: 261-268, 2012.

SCHULTZ, N. et al. Inoculation of sugarcane with diazotrophic bacteria. **Revista Brasileira de Ciência do Solo**, 38: 407-414, 2014.

SCHULTZ, N. et al. Yield of sugarcane varieties and their sugar quality grown in different soil types and inoculated with a diazotrophic bacteria consortium. **Plant Production Science**, 20: 366-374, 2017.

SCHULTZ, N. et al. Produtividade e diluição isotópica de 15N em cana-de-açúcar inoculada com bactérias diazotróficas. **Pesquisa Agropecuária Brasileira**, 51: 1594-1601, 2016.

SHUKLA, S. K. et al. Improving rhizospheric environment and sugarcane ratoon yield through bioagents amended farm yard manure in *udic ustochrept* soil. **Soil and Tillage Research**, 99: 158-168, 2008.

SILVA, M. A.; CATO, S. C.; COSTA, A. G. F. Produtividade e qualidade tecnológica da soqueira de cana-de-açúcar submetida à aplicação de biorregulador e fertilizantes líquidos. **Ciência Rural**, 40: 774-780, 2010.

SIMÕES, W. L. et al. Inoculação eficiente de bactérias diazotróficas via sistema de irrigação por gotejamento em cana-de-açúcar. **Pesquisa Agropecuária Brasileira**, 54: e01110, 2019.

URQUIAGA, S.; CRUZ, K. H. S.; BODDEY, R. M. Contribution of nitrogen fixation to sugarcane: nitrogen-15 and nitrogen balance estimates. **Soil Science Society of America Journal**, 56: 105-114, 1992.

VITTI, A.C. et al. Produtividade da cana-de-açúcar relacionada ao nitrogênio residual da adubação e do sistema radicular. **Pesquisa Agropecuária Brasileira**, 42: 249-256, 2007.